201-14820

October 30, 2003

Administrator
U S Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116
Attention: Chemical Right-to-Know Program

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RE: Registration number

Dear Sir/Madam:

International Flavors & Fragrances would like to submit the attached Test Plan and Robust Summary under the HPV Challenge Program, AR-201. This information pertains to the chemical:

Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl
CAS# 1222-05-5

The files attached are saved in Microsoft Word. We trust the information provided meets the requirements of the HPV Challenge Program. If you have any question or require further information, please contact me at 732-578-6724 or via e-mail at Uma.Parasar@iff.com

Sincerely,
For International Flavors and Fragrances, Inc.

Uma Parasar
Senior Toxicologist
Corporate Safety Assurance

cc: Dr. Paul Ribeiro, IFF
Dr. Mark Fukayama, IFF

201-14820A

Test Plan for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB) CAS# 1222-05-5

October 2003

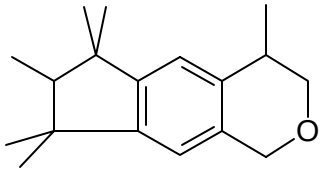
Submitted to the EPA under the HPV Challenge Program by:

International Flavors & Fragrances
1040 Broad Street
Shrewsbury, NJ 07703
Phone: 732-578-6700
Fax: 732-578-6756

Table of Contents

1 General Substance Information

1.1 Identity of Substances

CAS-No.:	1222-05-5
IUPAC name:	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (also CAS name)
Synonyms:	1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylindeno(5,6-c)pyran (EINECS name) HHCB Abbalide Chromanolide Pearlide Galaxolide
Molecular formula:	$C_{18}H_{26}O$
Structural formula: (main isomer)	
Molecular weight:	258.41

1.2 Introduction

HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran and related isomers) is used as an ingredient in the fragrance formulations of a wide variety of consumer products ranging from hydroalcoholic (typically in 70% ethanol) type products such as colognes and eau de toilettes to soaps and detergents.

HHCB consists of a main isomer and other minor structurally related isomers. It is a viscous material and is commonly sold and used as an approximately 65% dilution in a neutral solvent. Because this is the primary item of commerce, much of the safety testing has been conducted on the diluted material rather than the pure material.

Trace amounts of HHCB have been reported in human milk and fat samples as well as in the environment (Ford, 1998, Balk and Ford, 1999a). As a result, this material has been thoroughly tested for potential adverse effects in the environment and in humans. The results of these tests have been used to assess the risk to humans under the conditions of use in consumer products (Ford, 1998) and at the levels

found in the environment (Balk and Ford, 1999b). No additional testing of HHCB is anticipated under the HPV challenge program.

2.0 Physical properties

2.1 Melting point

Since HHCB is a mixture of isomers a lower and broader melting point is to be expected. The reported melting point is –10 - 0 °C (IFF, 2001).

2.2 Boiling Point

The boiling point of HHCB has been determined by calculation as well as by measurement. The boiling point for HHCB is 160 °C at 4 mm Hg as measured during the distillation of HHCB in the manufacturing plant (IFF, 2001). This conforms to the calculated value of 162 °C at 4 hPa using the Stein and Brown method.

2.3 Vapor Pressure

The measured vapor pressure for HHCB is 0.0727 Pa at 25 °C (Balk and Ford, 1999a).

2.4 Octanol/Water Partition Coefficients

The log Kow value for HHCB was determined according to OECD guideline no. 117 to be 5.9 (Balk and Ford, 1999a).

2.5 Water Solubility

The measured water solubility using 14C-labeled HHCB in three buffered solutions (pH 5, 7 and 9) by the flask method in accordance with OECD protocol 105 was determined to be 1.75 mg/L at 25 °C (Balk and Ford, 1999a).

2.6 Relative Density

The relative density of HHCB was determined to be 0.99 – 1.015 g/cm^3 at 20 °C using an oscillating densitometer and OECD Method 109 (IFF, 2001).

2.7 Flashpoint

The flashpoint of HHCB was determined to be > 100 °C using the closed cup, Pensky Martens Method (IFF, 2001).

2.8 New Testing Required

No new testing is required.

3 Environmental Fate

3.1 Photodegradation

The photodegradation of HHCB was studied by Aschmann et al. (2001) under laboratory conditions using black lamps for irradiation (λ > 300 nm) at 25 °C and 740 mm Hg (0.986 bar) total pressure of purified air at ~5% relative humidity. Photolysis and chemical reaction with OH radicals is the dominant atmospheric loss process. The measured rate constant for the gas phase reactions of OH radicals was $k_1 = 2.6 \pm 0.6 * 10^{-11}$ cm^3 $molecule^{-1}s^{-1}$, which agrees with the rate constant estimated from the structure of HHCB = $3.8* 10^{-11}$ cm^3 $molecule^{-1}s^{-1}$. Combined with estimated ambient atmospheric concentrations of OH radicals an atmospheric lifetime of 5.3 hours is calculated ($t_{½}$ = 3.7 h). The calculated lifetimes are inversely proportional to the assumed reactant concentration, and hence the lifetimes depend on time of day, season, and latitude. These data suggest that the atmospheric lifetime of HHCB is sufficiently short that it will not undergo long-range transport to any significant extent (Aschmann et al., 2001).

3.2 Stability in Water

Based on the chemical structure, HHCB is expected to be stable in water. There are no substituents subject to hydrolysis.

3.3 Biodegradation

Biotic degradation

Mineralization

The ready biodegradability of HHCB was assessed in (a) the sealed vessel headspace with total inorganic carbon analysis for CO_2-evolution and an adapted inoculum and (b) the modified Sturm test for CO_2-evolution (Balk and Ford, 1999a). In (a), HHCB was tested as a dilution in isopropyl myristate. The CO_2 evolved during the test was attributed solely to the biodegradation of isopropyl myristate. Test (B) was conducted on undiluted, viscous HHCB. Biodegradability was not observed. Both tests show the absence of mineralization under the stringent conditions of the tests for ready biodegradability. The tests are summarised in Table 3.3.

Table 3.3. Summary of tests for biodegradation (mineralization)

	Modification of OECD 301B, Sealed vessel TIC test acc. to Birch and Fletcher, 1991
Inoculum	Effluent from SCAS after 8 weeks adaptation
Test substance	HHCB in isopropyl myristate (commercially available quality), 10.97 mg C/l; 32.2%
Dispersion	Injection in isopropyl myristate
Test duration	28 days
Controls	Reference substance benzyl alcohol
	No toxicity control
Detection	TIC (Total Inorganic Carbon)
Results	% CO_2 release: zero (corrected for isopropyl myristate)
	Modified Sturm test OECD 301B, CO_2-evolution
Inoculum	sewage effluent, 1 drop/l
Test substance	HHCB, nominal 10 and 20 mg/l
Dispersion	No
Test duration	28 days
Controls	Reference substance Sodium benzoate; Toxicity control
Results	% CO_2 release: zero

Primary degradation

Though not readily biodegradable, HHCB has been demonstrated to degrade in the environment to more polar metabolites, with the lactone and the hydroxycarboxylic acid as likely intermediates. Primary degradation has been demonstrated in soil in the presence of common soil fungi (*Phanerochaete chrysosporium and Cladosporium cladosporiodes)* (Balk and Ford, 1999a and Van de Plassche and Balk, 1997).

The fate of 14C-HHCB in soil or sediment was studied in a microcosm study. Samples were taken from an oak forest soil, an agricultural soil and the sediment of the Delaware River in central New Jersey and from a farm with routine sludge applications from a domestic STP in southern New Jersey. Sealed flasks with soil spiked with 10 µg HHCB/g soil were incubated at laboratory ambient temperature for one year. For the four different soil types, an average of 14% HHCB remained after one year. Rate constants were 0.0066 d^{1} for sludge-amended soil, 0.0073 d^{1} for forest soil, 0.0029 d^{1} for agricultural soil and 0.0088 d^{-1} for river sediment. The estimated half-lives were 105, 95, 239 and 79 days, respectively. The average half-life in the four soils is 128 days (Balk and Ford, 1999a).

^{14}C-HHCB was dosed at 25 µg/l to activated sludge collected from three different STPs, and to river water (1 µg/l). The disappearance of the parent substance and the formation of metabolites were monitored over time. The half-life for the parent substance in activated sludge was determined to be 21 hours and in river water it was found to be 33 hours.

RP-HPLC analysis of the test media revealed that the metabolites in the activated sludge test (co-eluting with the lactone (log K_{ow} 4.0) and hydroxycarboxylic acid (log K_{ow} 0.5) standards) had lower Kows than the standards: from < 0.1 to 3.1. It was suggested that further oxidation of these products had

occurred. The capacity to metabolise HHCB was observed in all three STPs included in the study (Langworthy et al, 2000).

3.4 Fugacity

Transport and distribution in the environment were modelled using a Level III Fugacity Model through the EPA EPI Suite 2003 program. The input parameters used were molecular weight, molecular formula, water solubility, partition coefficient, and vapour pressure.

The model predicts that HHCB is distributed mainly to the sediment (55.6%) and soil (38.6%). The remaining material is distributed to water (5.58%) and air (0.188%).

Sediment concentrations of HHCB have been measured in European rivers. Chronological measurements have indicated a downward trend over time (HLUG, 2001). Primary degradation of HHCB in soil into more polar metabolites has been seen in experiments conducted with soil samples as discussed above (Balk and Ford, 1999a).

3.5 New Testing Required

No new testing is required.

4 Ecotoxicity

All studies in this section have been reviewed and used in environmental risk assessments in two recent publications (Balk and Ford, 1999a and 1999b).

4.1 Acute Toxicity to Fish

A 21-day prolonged toxicity test was carried out on HHCB with bluegill sunfish (*Lepomis macrochirus*) according to OECD Test Guideline 204 under flow-through conditions. The 21-d LC50 was 0.452 mg/l. The overall NOEC of the test was 0.093 mg/l as determined by the onset of clinical signs (Balk and Ford, 1999b; Wuthrich, 1996a).

4.2 Acute Toxicity to Invertebrates

For *Daphnia magna*, a semi-static 21-d toxicity test was carried out with HHCB according to OECD Test Guideline 202, part II, proposed updated version of June 1993 (Balk and Ford, 1999b; Wuthrich, 1996b). Under these conditions, the measured NOEC and LOEC were 0.111 and 0.205 mg/L, respectively and the 48 hr EC50 was 0.28 mg/L.

4.3 Acute Toxicity to Aquatic Plants

The toxicity of HHCB to algae was studied in a static test according to OECD Test Guideline 201 with *Pseudokirchneriella subcapitata*. Under the conditions of this test, the measured NOEC and LOEC were 0.201 and 0.466 mg/l, respectively and the EC50 for biomass production was 0.72 mg/L (Balk and Ford, 1999b; Van Dijk, 1997).

4.4 New Testing Required

No new testing is required.

5 Human Health Data

Most of the data in this section on HHCB have been reviewed and evaluated in a recent publication (Ford, 1998).

5.1 Acute Toxicity

Dermal

HHCB was applied to the skin of groups of 7 albino rabbits at a dose of 5 g/kg bw. The material as tested was a commercial sample and therefore, would have been approximately a 65% solution in a neutral solvent (private communication, IFF). Therefore the corrected dose administered was actually 3.25 g/kg bw. Since there were no deaths at that dose, the dermal LD50 was determined to be >3.25 g/kg bw.

An acute dermal limit test was also conducted on 5 female rats. No deaths were seen throughout the duration of the study. The LD50 was reported to be > 5 g/kg (Ford, 1998).

Oral

HHCB was administered to 10 rats at a dose of 5000 mg/kg bw followed by a 14-day observation. The material as tested was a commercial sample and therefore, would have been approximately a 65% solution in neutral solvent (private communication, IFF). Therefore, the corrected dose administered was actually 3.25 g/kg bw. A single mortality was observed throughout the14 day observation period. Therefore, the oral LD50 was determined to be >3.25 g/kg bw.

An acute oral limit test was conducted in female rats. Administration was by gavage and the rats were observed for 14 days. One death was seen at a dose of 3.25 g/kg. The LD50 is reported to be greater than 3.25 g/kg (Ford, 1998).

5.2 Genetic Toxicity

5.2.1 In Vitro

HHCB was tested in the Ames test (OECD guideline 471) both in absence and presence of Aroclor-induced rat liver S9 at doses ranging from 10 to 5000 µg/plate using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and Escherichia Coli strain WP2 UVRA. No significant increase in the number of revertant colonies was observed for HHCB at any dose with any of the six tester strains either in the presence or absence of activation (Api and San, 1999).

A second Ames test was conducted with HHCB in DEP using *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102 with and without rat liver S-9 (Aroclor 1254-induced) metabolic activation and with appropriate positive controls. The method used resembled OECD guideline 471. No significant increase in revertants was seen with HHCB at any dose (5-500 ug/plate) with or without activation (Mersch–Sundermann, et al. 1998a).

An *in vitro* micronucleus test was conducted with HHCB in DEP using human peripheral lymphocyte cultures obtained from healthy non-smoking donors aged 25-35 years. After induction of mitosis, HHCB (in DMSO) was added to the cultures with and without rat liver S-9 (Aroclor 1254 induced) metabolic activation for 48 hr. No significant increase in the frequency of micronuclei was seen with HHCB at concentrations up to 97 µM (194 µM was too cytotoxic to score) (Kevekordes, et al. 1997).

Another *in vitro* micronucleus test was conducted with HHCB in DEP using human hepatoma cells (Hep G2 line) which are capable of some metabolism. No significant increase in the frequency of micronuclei was seen with HHCB up to 194 µM (387 µM was too toxic to score) (Kevekordes, et al. 1997).

An *in vitro* unscheduled DNA synthesis (UDS) assay in accordance with OECD guideline 482 was conducted in primary rat hepatocytes. No increase in net nuclear grain count was seen for HHCB up to and including 15 µg/ml although this dose did induce significant cytotoxicity (50 µg/ml proved too toxic to be evaluated) (Api and San, 1999).

The ability of HHCB to induce sister-chromatid exchange (SCE) was evaluated using cultured human lymphocytes obtained from healthy non-smoking donors ranging in age from 25-35 years. The method used resembled OECD guideline 479. Concentrations of HHCB up to 48.5 ìM produced no effects (97 µM was too cytotoxic to be evaluated) (Kevekordes, et al. 1998).

A cytogenetic assay with Chinese Hamster ovary cells (CHO-K_1) was conducted according to OECD Guideline 473. The doses tested ranged from 9 – 30 ug/ml. HHCB was concluded to be negative for chromosome aberrations in this test (Api and San, 1999).

An SOS chromotest was conducted by incubating *Escherichia coli* PQ37 with HHCB with and without rat liver S-9 (Aroclor 1254 induced) metabolic activation. After a 2-hr incubation, enzyme activities of â-galactosidase and alkaline phosphatase were measured. Inducing factors, IF, were calculated relative to negative controls (solvent only). The tested doses ranged from 0.39 to 50 ug/assay. Both positive controls significantly increased IF but no inducing potency nor toxicity was seen with HHCB at any dose (Mersch-Sundermann, et al. 1998b).

5.2.2 In Vivo

HHCB was tested in a micronucleus test according to OECD guideline 474. The doses tested were 376, 750, and 1500 mg/kg (Api and San, 1999). No significant increase in micronucleated PCE in HHCB-treated groups relative to the respective vehicle control group was observed in male or female mice at 24, 48 or 72 hr after dose administration.

5.3 Repeat Dose Toxicity

A 13-week oral toxicity study in accordance with OECD guideline 408 and conforming to GLP was conducted in 150 rats CD (SD) (4 groups of 15 males and 15 females receiving HHCB by dietary admixture at 5, 15, 50, 150 mg/kg bw/day while a control group (15 males and 15 females) received the normal diet.

There were no mortalities or adverse clinical signs. Body weight and food consumption rates of treated groups were similar to those observed in the control group. No changes in ophthalmologic evaluation or significant histopathological findings were observed at any dose. The LOAEL in the study was based on the 2-week range finding study which was 347 mg/kg bw/day (increased liver weights seen at this dose). The NOAEL was determined to be 150 mg/kg bw/day (Api and Ford, 1999).

5.4 Reproductive Toxicity

HHCB was subjected to a peri- and post-natal development study where HHCB was administered by gavage to pregnant rats at daily doses up to 20 mg/kg bw starting in the third week of pregnancy and continuing through parturition until the weaning of the F1 generation. The F1 generation, which had been exposed to measurable levels in the milk (Hawkins and Ford, 1996), was allowed to grow to maturity and produce a third generation. There were no adverse effects on the F0 dams or the F1 and F2 offspring at the highest dose administered, 20 mg/kg bw/day. It should be noted that this dose cannot be considered as a NOAEL for the purpose of risk characterisation since it is the dose received by the dams and the study was designed to detect adverse effects on the pups.

This study was conducted in accordance with GLP and based on the guidelines endorsed by the ICH Steering Committee on the Detection of Toxicity to Reproduction for Medicinal Products (Ford and Bottomley, 1997, Jones et al, 1996).

5.5 Developmental Toxicity

HHCB was subjected to a developmental/teratology study in rats by administration of doses of 50, 150 or 500 mg/kg bw/day on days 7 through 17 of pregnancy. The maternal no-observable-adverse effects level (NOAEL) for HHCB was concluded to be 50 mg/kg bw. Based on a reduction in foetal body weight and an increased incidence of foetal skeletal (vertebral/rib) variations, the developmental NOAEL was 150 mg/kg bw.

This study was conducted in accordance with GLP and based on the guidelines endorsed by the ICH Harmonized Tripartite Guideline stages C and D (Christian, et al., 1999)

5.6 New Testing Required

No new testing is required

6 Test Plan Table

Physical-Chemical Properties				
Meting point	Boiling point	Vapor Pressure	Partition Coefficient	Water Solubility
A	A	A	A	A

Environmental Fate and Pathways			
Photo-degradation	Stability in water	Biodegradation	Fugacity
A	NA	A	Calc

Ecotoxicity		
Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
A	A	A

Human Health Data					
Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
A	A	A	A	A	A

Legend

A: End point requirement fulfilled with adequate existing data
NA: Not applicable due to physical/chemical properties
Calc: Endpoint requirement fulfilled based on calculated data

In conclusion, the data set on HHCB is robust and satisfies all the end points under the USEPA High Production Volume requirements. Therefore, no further testing is required on this material.

7 References

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Mersch-Sunderman, V, Kevekordes, S, and Jenter, C (1998a). Lack of mutagenicity of polycyclic musk fragrances in Salmonella Typhimurium. Tox. in Vitro, 12: 389-393.

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201-14820B

Robust Test Summaries for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran (HHCB) CAS# 1222-05-5

October 2003

Submitted to the EPA under the HPV Challenge Program by:

International Flavors & Fragrances
1040 Broad Street
Shrewsbury, NJ 07703
Phone: 732-578-6700
Fax: 732-578-6756

Table of Contents

Robust Summary for HHCB

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 Chemical and Physical Properties

1.1 Melting Point

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	Sample was cooled to –30° C and gradually warmed
GLP	No
Melting Point	-10 to 0 degrees C
Data Qualities Reliabilities	Reliability 2. Reliable with restriction
Remarks for Data Reliability	The substance is a mixture of isomers and is not expected to have a precise melting point.
References	IFF, 2001

1.2 Boiling Point

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	Calculated using Syracuse Research Corporation SAR-based software
GLP	No
Year	2000
Boiling Point	162° C @ 760 mm
Data Qualities Reliabilities	Reliability 4. Not assignable
Remarks for Data Reliability	Data calculated by recognized SAR program with input of log Kow, VP and water solubility.
References	William Meylan and Philip Howard, 2000. EPI Suite v 3.10, Syracuse Research Corporation

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Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	Measured during distillation
GLP	No
Year	2001
Boiling Point	160° C @ 4 mm Hg
Data Qualities Reliabilities	Reliability 2. Reliable with restriction
Remarks for Data Reliability	The measured boiling point was recorded in the distillation of HHCB in the manufacturing plant.
References	IFF, 2001

1.3 Vapor Pressure

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	OECD 104
Vapor Pressure	0.0727 Pa
Temperature	25° C
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and data are published in a peer-reviewed journal
References	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

1.4 n-Octanol/Water Partition Coefficient

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	OECD 117
GLP	Yes
Year	1999
Log Pow	5.9
Temperature	25° C
Remarks for Test Conditions	Conducted under GLP
Remarks for Results	Result is an average of 5.8 and 6.0, the values for the 2 principal isomers
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and data are published in a peer-reviewed journal.
References	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

1.5 Water Solubility

Substance Name	HHCB
CAS No.	1222-05-5
Method/Guideline	OECD 105
GLP	Yes
Year	1996
Value (mg/L) at Temperature	1.75 @ 25° C at a pH of 7
Remarks for Test Conditions	
Remarks for Results	Water solubility was 1.99 mg/L at a pH of 5 and 1.69 mg/L at a pH of 9.
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and data are published in a peer-reviewed journal
References	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

1.6 Relative Density

Substance Name	HHCB
CAS No.	1222-05-5
Method/Guideline	OECD 109
GLP	No
Year	2001
Value (mg/L) at Temperature	0.99 – 1.015 g/cm^3 @ 20° C
Remarks for Test Conditions	Measured with an oscillating densitometer.
Remarks for Results	
Data Qualities Reliabilities	Reliability 2. Reliable with restrictions
Remarks for Data Reliability	
References	IFF, 2001

1.7 Flashpoint

Substance Name	HHCB
CAS No.	1222-05-5
Method/Guideline	Pensky Martens Method (closed cup)
GLP	No
Year	2001
Value (mg/L) at Temperature	>100° C
Remarks for Test Conditions	
Remarks for Results	
Data Qualities Reliabilities	Reliability 2. Reliable with restrictions
Remarks for Data Reliability	
References	IFF, 2001

2 Environmental Fate and Pathways

2.1 Photodegradation

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Irradiation
Half-life t1/2	3.7 hours
Remarks for Test Conditions	Black irradiation lamps of λ >300 nm at 25° C and 740 mmHg.
Remarks for Results	
Data Qualities Reliabilities	Reliability 1. Data are reliable without restriction
Remarks for Data Reliability	Data obtained under laboratory conditions using methyl vinyl ketone as a reference substance.
References	Aschman SM, Arey J, Atkinson R and Simonich SL, 2001. Atmospheric lifetimes and fates of selected fragrance materials and volatile model compounds. Environmental Science and Technology, 359180, 3595-3600.

2.2 Biodegradation

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	HHCB undiluted (Purity 99%)
Method	OECD 301B
Test Type	Ready Biodegradability
GLP	Yes
Year	1994
Contact Time	28 days
Innoculum	Sewage effluent 1drop/L
Remarks for Test Conditions	Modified Sturm, $C0_2$ Evolution, Sodium benzoate as reference substance.
Degradation % After Time	0%

Time required for 10% degradation	
Remarks Results	HHCB is not mineralized in the ready biodegradability test.
Conclusion Remarks	Further tests have shown that HHCB is inherently biodegradable.
Data Qualities Reliabilities	Reliability 1. Data are reliable without restriction
Remarks for Data Reliability	Data generated using approved OECD protocol under GLP and also published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Commercial sample (HHCB in diluent isopropyl myristate)
Method	Modified OECD 301B
Test Type	Ready Biodegradability
GLP	Yes
Year	1993
Contact Time	28 days
Innoculum	Sewage effluent from SCAS after 8 weeks adaptation, 1drop/L
Remarks for Test Conditions	Sealed vessel Total Inorganic Carbon (TIC) test, Benzyl alcohol as reference substance.
Degradation % After Time	0% (corrected for isopropyl myristate)
Time required for 10% degradation	
Remarks Results	HHCB is not mineralized in the ready biodegradability test.
Conclusion Remarks	Further tests have shown that HHCB is inherently biodegradable.
Data Qualities Reliabilities	Reliability 1. Data are reliable without restriction
Remarks for Data Reliability	Data generated using approved OECD protocol under GLP and also published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Commercial sample (HHCB in diluent)
Method	
Test Type	Primary Degradation
GLP	No
Year	
Contact Time	
Remarks for Test Conditions	64 samples from different soil types were screened for the presence of naturally occurring micro-organisms. Pure cultures of fungi (Aureobasidium pullulans and Phanerochaete chrysosporium) were incubated with HHCB. Ethyl acetate extracts of the cultures were analysed by GC MS.
Conclusion Remarks	HHCB was demonstrated to degrade to more polar metabolites with the lactone and the hydroxycarboxylic acid as likely intermediates.
Data Qualities Reliabilities	Reliability 2. Data are reliable with restrictions
Remarks for Data Reliability	Data published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Radiolabeled HHCB
Method	
Test Type	Soil Microcosm primary degradation
GLP	
Year	1998
Contact Time	1 year
Remarks for Test Conditions	Samples were taken from 1) oak forest 2) agricultural field, 3) sediment of the Delaware river in central New Jersey and 4)

	sludge amended soil from a farm. Sealed flasks with soil spiked with 10 ug HHCB/g soil were incubated at laboratory temperatures for 1 year. Closed systems were used, with periodic flushing of headspace for oxygen replenishment and effluent gas was drawn through a train of scintillation fluids to capture volatiles and CO2. After the incubation period, the flasks were extracted with solvent and analysed for HHCB.
Conclusion Remarks	An average of 14% of HHCB remained in the soil after one year demonstrating a half-life value of 4 months for HHCB in soils.
Data Qualities Reliabilities	Reliability 2. Data are reliable with restriction
Remarks for Data Reliability	Data published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Radiolabeled HHCB
Method	
Test Type	Biotransformation
GLP	
Year	2000
Contact Time	
Remarks for Test Conditions	To understand the fate of HHCB in the environment, biotransformation was examined under realistic conditions in activated sludge and river water. Radiolabeled HHCB was dosed to freshly collected activated sludge (25 ug/L) and river water (1 ug/L). The disappearance of parent and the formation of metabolites were monitored over time.
Conclusion Remarks	The half-lives for parent HHCB were 21 hours in activated sludge and 33 hours in river water. HHCB is biotransformed in activated sludge and river water to polar metabolites that are predicted to be less bioaccumulative and less toxic than the parent compound. Therefore, concentrations of HHCB measured in the environment are lower than predicted concentrations.

Data Qualities Reliabilities	Reliability 2. Data are reliable with restriction
Remarks for Data Reliability	Data published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999a. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. I. Fate and Exposure Assessment. Toxicology Letters, 111, 57-79. Langworthy DE, Itrich NR, Simonich SL, and Federle TW, 2000. Biotransformation of the Polycyclic Musk HHCB in activated sludge and river water. Presented at SETAC, May 2000, Brighton, U.K.

2.3 Fugacity

Substance Name	HHCB
CAS No.	1222-05-5
Model Conditions	25° C, 100,000 lbs
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	EPT V 3.10 Level III
Input Parameters	MW, measured log Kow, measured water solubility and measured VP
Year	2003
Media	Air-Water Soil-Sediment-Partition Coefficient
Estimated Distribution and Media Concentration Model data and results	Air = 0.188%, Water = 5.58%, Soil = 38.6% and Sediment = 55.6%
Remarks	
Data Qualities Reliabilities	Reliability 4. Not assignable.
Remarks for Data Reliability	The data are obtained by a recognized fugacity calculation method. However, the method is an estimation.
References	USEPA, 2003 SRC EPIWIN Program

3 Ecotoxicity

3.1 Acute Toxicity to Fish

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Purity 99.15% isomeric mixture
Method/guideline	OECD 204
Test Type	21-day prolonged bluegill sunfish toxicity test
GLP	Yes
Year	1996
Species/Strain/Supplier	Lepomis macrochirus
Exposure Period	21 days
Analytical monitoring	Start, half-way through and at end.
Remarks for Test Conditions	Flow-through conditions. Nominal concentrations were 0.125 to 2.0 step size
Endpoint value	LC50 = 0.452, NOEC = 0.093, LC100=0.83, LOEC= 0.182
Unit	mg/L
Conclusion Remarks	Clinical signs included loss of equilibrium, irregular respiration and cessation of food intake. Results are expressed based on the mean measured concentrations of HHCB in the test which were 0.093, 0.182, 0.393, 0.830 and 1.566 mg/L.
Data Qualities Reliabilities	Reliability 1. Reliable without restriction
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and the data are published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999b. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. II. Effect Assessment and Risk Characterization. Toxicology Letters, 111, 81-94. Wuthrich, V. 1996a. HHCB: 21-day prolonged toxicity study in the bluegill sunfish under flow-through conditions. Report to RIFM., RCC Umweltchemie AG Project 380711.

3.2 Acute Toxicity to Aquatic Invertebrates

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Purity 99.15% isomeric mixture
Method/guideline	OECD 202
Test Type	Semi-static 21-day Daphnia
GLP	Yes
Year	1996
Species/Strain/Supplier	Daphnia magna
Analytical procedures	48-hour LC50 was calculated based on the 21-day test.
Test Details	Nominal concentrations ranged from 0.062 to 1.0 mg/l. Step size 2.
Remarks for Test Conditions	
EC50, EL50, LC0, at 24,48 hours	NOEC(rep) = 0.111, LOEC = 0.205, EC50 = 0.282 (48-hrs)
Unit	mg/L
Biological observations	
Remarks for Results	Results are based on the measured concentrations of HHCB in the test which were 0.049, 0.111, 0.205, 0.419, and 0.842 mg/L. 48-hour LC_{50} was calculated based on the 21-day test.
Data Qualities Reliabilities	Reliability 1. Reliability without restriction
Data Reliability Remarks	Study was conducted according to an OECD protocol under GLP and the data are published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999b. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. II. Effect Assessment and Risk Characterization. Toxicology Letters, 111, 81-94. Wuthrich, V. 1996b. Influence of HHCB on the reproduction of Daphnia magna. Report to RIFM. RCC Umweltchemie AG Project 380687.

3.3 Acute Toxicity to Aquatic Plants

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	OECD 201
Test Type	Algae Static growth inhibition test
Species/Strain/Supplier	Pseudokirchneriella subcapitata
Exposure Period	72 hours
Remarks for Test Conditions	Endpoint was growth rate as well as biomass. Start concentrations were 71-102% of nominal and end concentrations 54-85% of nominal. Mean measured concentrations were 0.042, 0.084, 0.201, 0.466 and 0.844 mg/L. Nominal concentrations ranged from 0.065 to 1.0 mg/L. Step size 2. Results are based on the mean measured concentrations.
Endpoint value	NOEC = 0.201, LOEC = 0.466, EC50 for biomass production = 0.72 mg/L, EC50 for growth = > 0.854 mg/L.
Conclusion Remarks	
Data Qualities Reliabilities	Reliability 1. Reliability without restriction
Remarks for Data Reliability	Study conducted according to an OECD protocol under GLP and the data are published in a peer-reviewed journal.
Reference	Balk F and Ford RA, 1999b. Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU. II. Effect Assessment and Risk Characterization. Toxicology Letters, 111 81-94. Van Dijk, A. 1997. Acute toxicity of HHCB to Pseudokirchneriella subcapitata. Report to RIFM, RCC Umweltchemie AG Project 380632.

4 Human Health Toxicity

4.1 Acute Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Acute oral toxicity limit test
GLP	Pre-GLP
Year	1975
Species/strain	Rats, Wistar
Sex	Male and Female
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Gavage
Remarks for Test Conditions	14-day observation period
Value LD50 or LC50 with confidence limits	> 3.25 g/kg
Number of deaths at each dose level	1/10 at 3.25 g/kg
Remarks for Results	The material as tested was a commercial sample and therefore, would have been an approximately 65% solution. Therefore the dose administered has been corrected from 5g/kg to 3.25 g/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Moreno, O.M. 1975. Galaxolide 50: acute oral toxicity in rats; dermal toxicity in rabbits. Project No. MB 75-770. MB Research Report to the Research Institute for Fragrance Materials, Inc. (RIFM). Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Acute oral toxicity limit test
GLP	Pre-GLP
Year	1977
Species/strain	Rats
Sex	Female
# of animals per sex per dose	10
Vehicle	None
Route of Administration	Gavage
Remarks for Test Conditions	14-day observation period
Value LD50 or LC50 with confidence limits	> 3 g/kg
Number of deaths at each dose level	0 at highest dose
Remarks for Results	The material as tested was a commercial sample and therefore, would have been an approximately 65% solution.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Acute dermal toxicity limit test
GLP	Pre-GLP
Year	1975
Species/strain	New Zealand white rabbits
Sex	Not reported
# of animals per sex per dose	7
Vehicle	None
Route of Administration	Dermal
Remarks for Test Conditions	14-day observation period
Value LD50 or LC50 with confidence limits	> 3.25 g/kg
Number of deaths at each dose level	0/10 at 3.25 g/kg
Remarks for Results	The material as tested was a commercial sample and therefore, would have been an approximately 65% solution. Therefore, the dose administered has been corrected from 5g/kg to 3.25 g/kg bw. There were no deaths at that dose. Therefore, the LD50 can be listed as >3.25 g/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Moreno, O.M. 1975. Galaxolide 50: acute oral toxicity in rats; dermal toxicity in rabbits. Project No. MB 75-770. MB Research Report to the Research Institute for Fragrance Materials, Inc. (RIFM). Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

Substance Name	HHCB
CAS No.	1222-05-5
Method/guideline	
Test Type	Acute dermal toxicity limit test
GLP	Pre-GLP
Year	1977
Species/strain	CRL Sprague-Dawley
Sex	Female
# of animals per sex per dose	5
Vehicle	Ethanol
Route of Administration	Dermal
Remarks for Test Conditions	7-day observation period
Value LD50 or LC50 with confidence limits	> 5 g/kg
Number of deaths at each dose level	0/5 at 5 g/kg
Remarks for Results	
Data Qualities Reliabilities	Reliability code 2. Reliable with restrictions
Remarks for Data Reliability	Data collected prior to GLP by method comparable to present guidelines/standards.
References	Ford RA, 1998. The human safety of the polycyclic musks, AHTN and HHCB in fragrances – A review, Dtsch, Lebens. Rdsch., 98(8), 268-275.

4.2 Genetic Toxicity

4.2.1 In vitro Genotoxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample supplied by IFF. Purity > 99% based on isomeric mixture.
Method/guideline	OECD 471
Test Type	Ames reverse mutation assay
System of Testing	
GLP	Yes
Year	1999
Species/Strain	Salmonella typhimurium TA98, TA100, TA1535, TA1537 and TA1538; Escherichia coli WP2 uvrA
Metabolic Activation	With and without S9 activation
Doses/Concentration	10, 33, 100, 333, 1000 or 5000 ug per plate
Statistical Methods	
Remarks for Test Conditions	All positive controls gave positive responses to the systems within acceptable ranges.
Results	No significant increase in the number of revertant colonies was observed with HHCB at doses of 10-5000 ug/plate.
Cytotoxic concentration	None
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	Ames reverse mutation assay
System of Testing	
GLP	Not reported
Year	1998
Species/Strain	Salmonella typhimurium TA97, TA98, TA100 and TA102
Metabolic Activation	With and without S9 activation
Doses/Concentration	5-500 ug per plate (corrected concentrations range from 3.25 to 325 ug/plate based on testing of 65% Galaxolide)
Statistical Methods	
Remarks for Test Conditions	The doses were 5, 16.6, 50, 166.6 or 500 ug/plate (limit of solubility)
Results	No significant increase in the number of revertant colonies was observed with HHCB at doses of 5-500 ug/plate.
Cytotoxic concentration	None
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Mersch-Sundermann V, Kevekordes S and Jenter C, 1998a. Lack of mutagenicity of polycyclic musk fragrances in Salmonella typhimurium. Tox. In Vitro, 12: 389-393.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	In vitro micronucleus
System of Testing	
GLP	No
Year	1997
Species/Strain	Human peripheral lymphocytes from healthy non-smoking donors
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.05, 0.49, 4.85, 48.5, 97 micromolar. (corrected for 65% solution = 0.0325, 0.3185, 3.152, 31.52, 63.05, 126.1 uM)
Statistical Methods	
Remarks for Test Conditions	Positive controls significantly increased the frequency of micronuclei.
Results	No significant increase in the frequency of micronuclei
Cytotoxic concentration	194 micromolar
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Kevekordes S, Mersch-Sundermann V, Diez M and Dunkelberg H, 1997. In vitro genotoxicity of polycyclic musk fragrances in the micronucleus test. Mutation Research, 395 (2-3), 145-150.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	In vitro micronucleus
System of Testing	
GLP	No
Year	1997
Species/Strain	Human hepatoma cells
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.1, 0.97, 9.7, 97, 194, 387 micromolar (corrected for 65% solution = 0.065, 0.6305, 6.305, 63.05, 126.1, 251.5 uM)
Statistical Methods	
Remarks for Test Conditions	Incubation period was 2 hours after which, the cells were harvested and scored for micronuclei.
Results	No significant increase in the frequency of micronuclei was seen with HHCB treatment.
Cytotoxic concentration	387 micromolar
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Kevekordes S, Mersch-Sundermann V, Diez M and Dunkelberg H, 1997. In vitro genotoxicity of polycyclic musk fragrances in the micronucleus test. Mutation Research, 395 (2-3), 145-150.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample supplied by IFF. Purity > 99% based on isomeric mixture.
Method/guideline	OECD 482
Test Type	In vitro unscheduled DNA synthesis
System of Testing	
GLP	Yes
Year	1999
Species/Strain	Primary rat hepatocytes from Sprague-Dawley rats
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.15, 0.50, 1.5, 5, 15, 50 ug/ml
Statistical Methods	
Remarks for Test Conditions	Positive control induced a significant increase in the average net nuclear grain count over controls.
Results	No significant increase in UDS
Cytotoxic concentration	50 ug/ml
Genotoxic Effects	None
Conclusion Remarks	No genotoxic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported but similar to OECD 479
Test Type	Sister-chromatid exchange (SCE)
System of Testing	
GLP	Not reported
Year	1998
Species/Strain	Human lymphocytes obtained from healthy non-smoking donors
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.025, 0.25, 2.43, 24.25, 48.5 or 97 micromolar (corrected for 65% solution = 0.0162, 0.1625, 1.579, 15.76, 31.52, 63.05 uM)
Statistical Methods	
Remarks for Test Conditions	Treatment time was 2 hours. Positive controls showed a significant increase in SCEs.
Results	No significant increase in the number of sister chromatid exchanges was observed with HHCB at the doses tested compared to non-treated lymphocytes.
Cytotoxic concentration	97 micromole
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Kevekordes S, Mersch-Sundermann V, Diez M, Bolten C and Dunkelberg H, 1998. Genotoxicity of polycyclic musk fragrances in the Sister-Chromatid Exchange test. Anticancer Research, 18: 449-452.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample supplied by IFF. Purity > 99% based on isomeric mixture.
Method/guideline	OECD 473
Test Type	Chormosome aberration with multiple harvest times
System of Testing	
GLP	Yes
Year	1999
Species/Strain	Chinese hamster ovary cells
Metabolic Activation	With and without S9 activation
Doses/Concentration	Wo/activation for 4/20, 20/20, 44/44 hr exposure/harvest (e/h) times at 5, 10, 20 microgram/ml; w/activation for 4/20 hr e/h with 9, 17, 34 microgram/ml and for 4/44 hr e/h with 23, 28, 30 microgram/ml
Statistical Methods	
Remarks for Test Conditions	Cells were assessed for structural chromosome aberrations at the 20 and 44-hr harvest time. Numerical chromosome aberrations were also assessed at the 44-hr harvest time.
Results	No significant increase in structural or numerical chromosome aberrations
Cytotoxic concentration	20 ug/ml w/o activation; 30 ug/ml with activation
Genotoxic Effects	None
Conclusion Remarks	No genotoxic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Material was obtained from a commercial source, Promochem, under the trade name Galaxolide. Galaxolide is marketed as a 65% solution in diethyl phthalate.
Method/guideline	Not reported
Test Type	In vitro
System of Testing	SOS induction using E. coli PQ37
GLP	No
Year	1998
Species/Strain	Human hepatoma cells
Metabolic Activation	With and without S9 activation
Doses/Concentration	0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25 or 50 ug (corrected for 65% solution = 0.25, 0.507, 1.014, 2.03, 4.06, 8.12, 16.25, and 32.5 ug)
Statistical Methods	
Remarks for Test Conditions	Incubation period was 2 hours after which enzyme activities of beta-galactosidase and alkaline phosphotase was measured.
Results	Both positive controls significantly increased in inducing factors (IF) but no inducing potency nor toxicity was seen with HHCB at any dose.
Cytotoxic concentration	
Genotoxic Effects	None
Conclusion Remarks	No mutagenic potential
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer-reviewed journal.
References	Mersch-Sundermann V, Kevelordes, S., and Jenter, C. 1998b. Testing of SOS induction of Artificial Polycyclic Musk Fragrances in E. coli PQ37. Toxicology Letters, 95: 147-154.

4.2.2 In vivo Genotoxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample > 99% purity based on isomeric mixture.
Method/guideline	OECD 474
Test Type	In Vivo mouse micronucleus cytogenetic assay
GLP	Yes
Year	1999
Species/Strain	ICR mice
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/Concentration	376, 750 or 1500 mg/kg
Exposure Period	Bone marrow cells were harvested and evaluated 24, 48 and 72 hours after dosing.
Remarks for Test Conditions	Negative control was corn oil and positive control was cyclophosphamide
Appropriate statistical evaluations?	
Effect on mitotic index or PCE/NCE ratio by dose level and sex	Moderate reductions (up to 25%) in the ratio of PCE to total erythrocytes were observed in groups on 1500 mg/kg bw after 48 and 72 hours indicating toxicity and bioavailability to the bone marrow.
Genotoxic effects	None
NOEL (C)/ LOEL (C)	
Remarks for Results	
Conclusion Remarks	No significant increase in micronucleated PCE in HHCB-treated groups relative to the respective vehicle control group was observed in male or female mice at 24, 48 or 72 hours after dose administration.
Data Qualities Reliabilities	Reliability 1. Reliable without restrictions.
Remarks for Data Reliability	Data are by a standard method and have been published in a peer reviewed journal.
References	Api, AM and San, RHC, 1999. Genotoxicity Tests with 6-Acetyl-1,1,2,4,4,7-hexamethyltetraline and 1,3,4,6,7,8-Hexahydro-

4,6,6,7,8,8-hexamethylcyclopenta-gamma-benzopyran. Mutation Research, 446: 67-81.

4.3 Repeat dose Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Colorless viscous liquid sample > 99% purity based on isomeric mixture.
Method/guideline	OECD 408
GLP	Yes
Year	1999
Species/strain	Crl:CD(SD)Br
Sex	Male and Female
Route of Administration	Diet
Doses/concentration Levels	5, 15, 50 or 150 mg/kg per day; 15M and 15F per dose
Exposure Period	13 weeks
Frequency of Treatment	Daily
Control Group	Diet only
Post Exposure	4 weeks post exposure observance for selected rats from control and high dose groups
Remarks for Test Conditions	HHCB was added to the diet to the desired concentration. The mean achieved daily intakes were 5.4, 15.7, 51.8 and 155.8 mg HHCB/kg bw for males and 5.1, 15.6, 51.9 and 154.6 mg HHCB/kg bw for females.
NOAEL (NOEL)	150 mg/kg
Toxic Response/effects by Dose Level	LOAEL based on 2 week range finding study = 347 mg/kg (increased liver weights seen at this dose)
Statistical Evaluation	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Study exceeded requirements of OECD 408 and are published in a peer reviewed journal.
References	Api, AM and Ford, RA, 1999. Evaluation of the Oral Subchronic Toxicity of HHCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran) in the Rat.

Toxicology Letters, 111: 143-149.

4.4 Reproductive Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Viscous neat material, purity > 95% supplied by IFF.
Method/guideline	International Conference on Harmonisation (ICH) Guideline on detection of Toxicity to Reproduction for Medicinal Products, endorsed by the ICH Steering Committee Step 4 of the ICH process. 24 June, 1993. Section 4.1.2
Test Type	Peri-and post-natal development
GLP	Yes
Year	1996
Species/Strain	Crl:CD(SD)Br
Sex	Female
Route of Administration	Oral gavage
Duration of Test	19 weeks
Doses/Concentration	2, 6 and 20 mg/kg/day. F1 offspring only exposed *in utero* or through mother's milk from birth to weaning.
Control Group and Treatment	Untreated
Frequency of Treatment	Daily
Remarks for Test Conditions	F0 were evaluated for behavioral effects and for reproductive effects resulting in an F2 generation
NOAEL(NOEL)	There is no NOAEL established in the study.
Appropriate statistical evaluations	
Parental data and F1 as Appropriate	No effects on F0 females. No effects of F1 males or females.
Remarks for Results	Exposure to test material by gavage to dams had no effects at any dose and exposure to F1 offspring through mother's milk had no effects on behavior or reproductive performance. F2 pups were without adverse effects. 20 mg/kg bw/day cannot be considered as the NOAEL for the purpose of risk characterization since it is the dose received by the dams and the study was designed to detect adverse effects on the pups.
Data Reliabilities Qualities	Reliability code 1. Reliable without restrictions.
Remarks for Data Reliability	Study conducted according to a recognized guideline and under GLP.
References	Ford, RA and Bottomley, A, 1997. A Method for Evaluation of the Potential Toxicity to the Neonate from Exposure to

Xenobiotics via Mother's Milk – Application to Three Fragrance materials. The Toxicologist 36, No.1, Part 2:367.

Jones, K., Bottomley A.M. and Gopinath, C. (1996) HHCB: Effects on peri- and post natal development including maternal function in the rat (Gavage administration). Report to RIFM. September, 1996

4.5 Developmental/Teratogenicity Toxicity

Substance Name	HHCB
CAS No.	1222-05-5
Remarks for Substance	Viscous neat material, purity > 95% supplied by IFF.
Test Type	Developmental toxicity
GLP	Yes
Year	1999
Species/strain	Rat/Crl:CD(SD)Br VAF/Plus (Sprague-Dawley)
Sex	Female
Route of Administration	Oral gavage in corn oil
Duration of Test	3 weeks
Doses/concentration Levels	50, 150 and 500 mg/kg/day
Exposure Period	Days 7 through 17 of pregnancy
Frequency of Treatment	Daily
Control Group and Treatment	Corn oil only
Remarks for Test Conditions	The study was conducted in accordance with ICH Harmonized Tripartite Guideline Stages C and D.
NOAEL(NOEL) maternal toxicity	50 mg/kg/day
LOAEL(LOEL) maternal toxicity	150 mg/kg/day
NOAEL (NOEL) developmental toxicity	150 mg/kg/day
LOAEL developmental Toxicity	500 mg/kg/day
Actual dose received by dose level and sex	
Maternal data with dose level	Reduction in maternal bodyweight gain and feed consumption at two highest doses.

Fetal Data with Dose Level	Decreased mean bodyweights with axial skeleton (vertebral/rib) variations increased in high dose group only.
Appropriate statistical evaluations	
Remarks for Results	Material was not more toxic to the conceptus than to the dam.
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restrictions.
Remarks for Data Reliability	Study conducted according to a recognized guideline under GLP and is published in a peer-reviewed journal.
References	Christian MS, Hoberman AM, Diener, RM, Parker RM and Api, AM (1999). Developmental toxicity study of four fragrances in rats. Toxicology Letters, 111: 169-174.